Survey of non-polar extractables (bio-crude) from *Grindelia ciliata* (ASTERACEAE: Astereae)

Robert P. Adams and Amy K. TeBeest
Biology Department, Baylor University, Waco, TX 76798, USA
Robert_Adams@baylor.edu

Judy Etling
360 Bullock Road
Newcastle, TX 76372

Walter Holmes
Biology Department, Baylor University, Waco, TX 76798, USA

and

Nathan Taylor
Biology Department, Sul Ross State University, Alpine, TX

ABSTRACT

Non-polar (pentane) extractables (bio-crude) were examined from natural populations of *Grindelia ciliata* from Texas, Oklahoma and Kansas. The highest yielding populations were from ne Amarillo, TX (14.79%), Childress, TX (13.53%) and Bullock Rd., Newcastle, TX (12.84%). The lowest yielding populations were from Spearman, TX (6.78%) and Cimarron River, KS (6.72%). The most variable populations were: Lake Tanglewood (LT, 20.7%), Childress (CAT, 19.56%) and Waco, TX (WCO, 18.6%). The populations with least variation were: Borger, TX (BOR, 5.5%), Lamesa, TX (LAM, 7.6%) and McGregor, TX (McG, 8.8%). The yields from 166 individuals screened ranged from 3.89 to 18.92%. The top five percent of the plants (8) ranged from 15.76 to 18.92%, indicating a good pool of plants for improvement of yields. The optimum time for maximum bio-crude yields was not determined, and appears to vary by elevation and length of growing season. Published on-line www.phytologia.org *Phytologia 98(1): 30-36 (Jan 5, 2016)*. ISSN 030319430.

KEY WORDS: *Grindelia ciliata,* Asteraceae, non-polar extractables, bio-crude, geographic variation, Spanish Gold, giant gumweed.

Grindelia (gumweed) is a large genus of about 75 species with an amphitropical distribution with half of the species occur in North America and Mexico and the remaining species in South America (Moore, et al., 2012). Steyermark (1934) recognized 45 species, plus 40 varieties and 25 forms (110 taxa). Strother and Wetter (2006) estimated the genus to contain some 30 species; they recognized only 18 species in the Flora of North America. Bartoli and Tortosa (2012) recognized 41 species, 10 varieties and 2 forms in North America based on classical morphology. Moore et al. (2012) utilized nrDNA and the ETS region as well as psaI-accD cpDNA to analyze selected taxa of *Grindelia* from both North and South America. They found strong support for two sister clades in North and South America. The North American clade seemed to be divided into two groups by the continental divide.

Nesom, Suh and Simpson (1993) submerged the monotypic genus *Prionopsis* (*P. ciliata*) into *Grindelia* as *G. ciliata* (Nuttall) Sprengel. [syn: *G. papposa* (Nutt.) Nesom & Suh]. *Grindelia ciliata* reportedly grows as an annual or biennial species. It is widely distributed in Texas, Oklahoma and Kansas, se Colorado, e New Mexico, s Nebraska, s and se Iowa (rare) with putative outlying records from Illinois, Missouri, Arkansas and Louisiana. Distribution reports from Arizona and California are problematic. Seinet (2015) maps a record (*G. papposa* = *G. ciliata*) in nw Michigan, but the specimen is *Jeff Hansen sn,* Horsethief Canyon, Ellsworth, KS, 30 Sep 2009 and is obviously mis-mapped.

Grindelia has been the subject of several studies on its use as a 'bio-crude' species (Adams et al. 1986; Hoffmann and McLaughlin, 1986; McLaughlin and Linker, 1987) and the genus is well known for its diterpene acids (Timmermann et al., 1987) which can be used as a substitute for pine resins in the plastics and paper industries as well, as cracked and re-formed for use as fuel (bio-fuel). Much of the work by the Arizona group was based on *G. camporum* Greene, native to central California. McLaughlin and Hoffmann (1982) reported cyclohexane (non-polar) yields from: *G. aphanactis* (= *G. nuda* var. *aphanactis*): 7.2%; *G. camporum*: 5.5 - 13.0%; *G. robusta*: 8.4%; and *G. squarrosa*: 8.8 - 13.8%. Adams et al. (1986) reported hexane (non-polar) yields from: *G. acutifolia* (CO): 5.0 - 10.23%; *G. aphanactis* (NM): 7.95%; *G. arizonica* var. *stenophylla* (CO): 8.36%; *G. decumbens* (CO): 7.11%; *G. fastigiata* (CO): 7.47 - 9.62%; *G. nana* (WA): 10.36%; *G. squarrosa*: ND - 9.65%, WA - 9.04 - 11.67%, NM - 10.23%, CO - 7.16 - 11.12%; and *G. subalpina* var. *erecta* (CO): 8.62%.

Grindelia ciliata is a large plant (up to 2 m), that grows in disturbed sites in various soils and precipitations (Figs. 1, 2). It appears to have potential as a semi-arid land bio-crude crop plant. In contrast to most *Grindelia* species, in *G. ciliata*, the leaves and buds are not gummy or with exuded resin, yet, the bio-crude yields are comparable to sticky or gummy *Grindelia* species. Sequestering the resin inside the leaves would seem to be an important agronomic character, as sticky leaves would be difficult to swath and bale. The purpose of the present paper is to survey non-polar (pentane) yields from individuals in natural populations of *G. ciliata* to evaluate its potential as a source of bio-crude.

Fig. 1. Close-up of *G. ciliata* flower and buds. Grimes Co., TX, Photo by Nathan Taylor.

Fig. 2. *G. ciliata* population on Bullock Rd., Newcastle, TX. Photo by Judy Etling.

MATERIALS AND METHODS

Fresh leaves and specimens of *G. ciliata* were collected from the following populations:

1. LT, Lake Tanglewood, Amarillo, TX 35° 04' 35" N, 101° 47' 24" W, 3596 ft. at telephone tower, most plants branched, in prairie sod (buffalo grass), loam soil. 13 Aug 2015, LTP1-10,
2. SP, Spearman, Tx, 36° 11' 16" N, 100° 48' 32" W, 2949 ft, common along caliche road (Co. Rd. W), and along hwy US 83 to Canadian, TX, most plants with single stems, caliche soil on road side, 1 mi east of Jct. Tex 70 & Farm Rd. 759 on Co. Rd. W. 15 Aug 2015, SP1-10,

3. CAT, Canadian, TX, 11 mi. east of US 83 on Tex 2266. 35° 53' 22" N, 100° 02' 05" W, 2205 ft., occasional, most plants with single stems, in deep sand, lots of small black (sugar?) ants on heads. 15 Aug 2015, CAT1-10,
4. BO, 9 mi N of OK/TX border, on US83, at a ravine on terrace n of Beaver River, mostly single stemmed, locally common on sandy soil. 36° 35' 14" N, 100° 49' 42" W, 2893 ft, 19 Aug 2015, *Adams 14631*, BO1-10,
5. CK, 3 mi N of KS/OK border on KS hwy 23, n of Cimarron River, locally common, mostly single stemmed, on sandy soil. 37° 01' 27" N, 100° 29' 39.5" W, 2378 ft, 19 Aug 2015, CK1-10C,
6. DCK, on US 56, 3 mi w of jct US 56 US 54, on sandy soil in ditch, Dodge City, KS, scattered but locally common, mostly single stemmed, 37° 43' 13" N, 100° 04' 11" W, 2510 ft, 19 Aug 2015, DCK1-10,
7, 8. FH, Fritch Highway, about 17 mi ne of Amarillo, TX, 35° 25' 34" N, 101° 38' 07" W, 3520 ft. on Tex 136, most plants branched. Common on west side of hwy from this locatioin near Fritch, TX (to last ravine) on Tex 136. in prairie grass, loam soil. 21 Aug 15, recollection from same tagged plants, 3 Sept 2015, FHR1-10,
9. BOR, 1 mi s of Borger, TX on Tex 207 on road cut, sandy but caliche on top, mostly branched plants. 35° 38' 17" N, 101° 23' 50" W, 3203 ft, 22 Aug 2015. *Adams 14636*, BOR1-10,
10. AIR, few hundred plants, most single stemmed. on poor soil, mostly caliche, with sunflowers and disturbed prairie. Air Products Plant, n on Tex 136, ~1 mi s of OK border, 36° 29' 19" N, 101° 28' 20" W, 3156 ft., 1 Sept 2015, *Adams 14638*, AIR-10,
11. PST, ~5 mi w of Post, TX, on US 84 and CR 165, 20-30 plants, disturbed site, on sand, 33° 13' 02" N, 101° 26' 02" W, 2939 ft., 27 Aug 2015, PST1-5,
12. SAT, to 6 ft tall, spindly, 20-30 plants, on vacant lot, sand, on US 183 in Santa Ana, TX, 31° 44' 32" N, 99° 19' 51" W, 1725 ft., 27 Aug 2015, *Adams 14640*, SAT1-5,
13. BR, around oil tanks, on red loam, half plants were branched, on Bullock Road, near Newcastle TX, 33° 09' 34" N, 98° 41' 54" W, 1217 ft., 30 Aug 2015, *Adams 14642*, BR1-10,
14. CHD, on vacant lot in Childress, red sand, 100 plants, many branched, on US 287, 34° 24' 47" N, 100° 10' 02" W, 1737 ft, 30 Aug 2015, *Adams 14644*, CHD1-10,
15. GRU, on caliche, disturbed prairie, ~100 plants, most single stemmed, ~20% flowers seeded, Hansford Gun Club, 36° 12' 38" N, 101° 18' 59" W, 3145 ft, 1 Sept 2015. *Adams 14645*, GRU1-10,
16. McG, on vacant lot, sandy-loam, 10 plants, on US84, 7 m n of McGregor, 8 mi. s of Waco, TX, 31° 28' 48" N, 97° 17' 35" W, 540 ft., 27 Aug 2015, *Adams 14641*, MCG1-6,
17. WCO, right-of way, Tex. 3400 at Flat Creek, 4.5 miles nw of Robinson, on highly disturbed alluvium consisting of clay to silt. Disturbance is mostly due to grading to build up the edge of right-of-way to limit drainage from adjoining agricultural fields and periodic cutting. Population of about 75-100 plants spread over about 100 m (at the outer edge of right-of-way). Plants 1-1.5 m tall, probably small due to drought. 31° 29' 30.43" N, 97° 04' 51.25" W, 400 ft.,23 Aug 2015. *W. Holmes ns*, Lab. Acc. *Adams 14646*, WCO1-10,
18. LMT Lamesa, TX population, 3-4 mi. w of the Gaines-Dawson Co. line about a mile south of 180. 32°41'07" N, 102°15'51" W, elev. 3063 ft., *Nathan Taylor ns*, 20 Aug 2015, LMT1-10.

Pentane non-polar extraction - Leaves were collected from branched plants, so we could remove leaves from 4 branches without seriously damaging the health of the plant. Four side branches (each approximately 30 cm long) were cut and the fresh leaves stripped into a paper bag. Upon return to the lab, the bags were air dried (40 - 42°C) for 24 h (7.8% moisture). The leaves were ground in a coffee mill to pass a 1 mm screen. Three (3) g. of air-dried leaves were extracted with 19 ml of pentane on a platform shaker for 24 h. The supernatant was filtered into a pre-weighed aluminum disposable pan. The extracted material (marc) was washed with 2 ml of pentane and the supernatant filtering into the aluminum pan. The pentane was evaporated (40°C) in a hood. The pan, with extract, was weighed and the tare weight subtracted.

Paired extractions (3) using pentane-shaker (24 h) vs. pentane-soxhlet extraction (8h, until no color was being removed) revealed that the pentane-soxhlet extraction resulted in 2.1x higher yields than pentane-shaker extractions. Because pentane-shaker extractions can be done in large numbers, this method was chosen and the yields corrected by a 2.1 factor. The final shaker yields were corrected to an oven dry weight (ODW) basis by post-multiplication of correction factor (CF=2.29), where CF = 2.11 (soxhlet/shaker yields) x 1.085 (air dried wt./ ODW) = 2.29.

ANOVA was computed for percent crude oil and means tested for significance using Student-Newman-Keuls (SNK) multiple range tests of significance, computed by use of program SNK, written by RPA, using formulation Steel and Torrie (1960). Pearson Product Correlation was computed by use of http://www.socscistatistics.com/tests/pearson/Default2.aspx.

RESULTS AND DISCUSSION

The yields of bio-crude varied considerably within and among populations (Table 1). The highest yielding populations were ne Amarillo (14.79%), Childress (13.53%) and Bullock Rd. (12.84%) (Table 1). The lowest yielding populations were Spearman (6.78%) and Cimarron River, KS (6.72%) (Table 1). To visualize the geographic variation, yields were contour mapped (Fig. 3). It is interesting that the highest yielding populations form a nw to se line (FH, CHD, BR). Yields in most of the other populations range from 6.72 to 9.12%, except the SAT population (10.6%). Of course, this is the product of both genes and the environment. It might be noted that this year (2015) was the 5th wettest year in recorded history at Amarillo (~32"). However, the difference between nearby populations FH (14.8%) and BOR (7.7%) seems likely genetic, and not due to changes in habitat. The populations in ne Texas Panhandle, Oklahoma and sw Kansas are all low in bio-crude. The populations from west Texas and central Texas tend to be higher in yields.

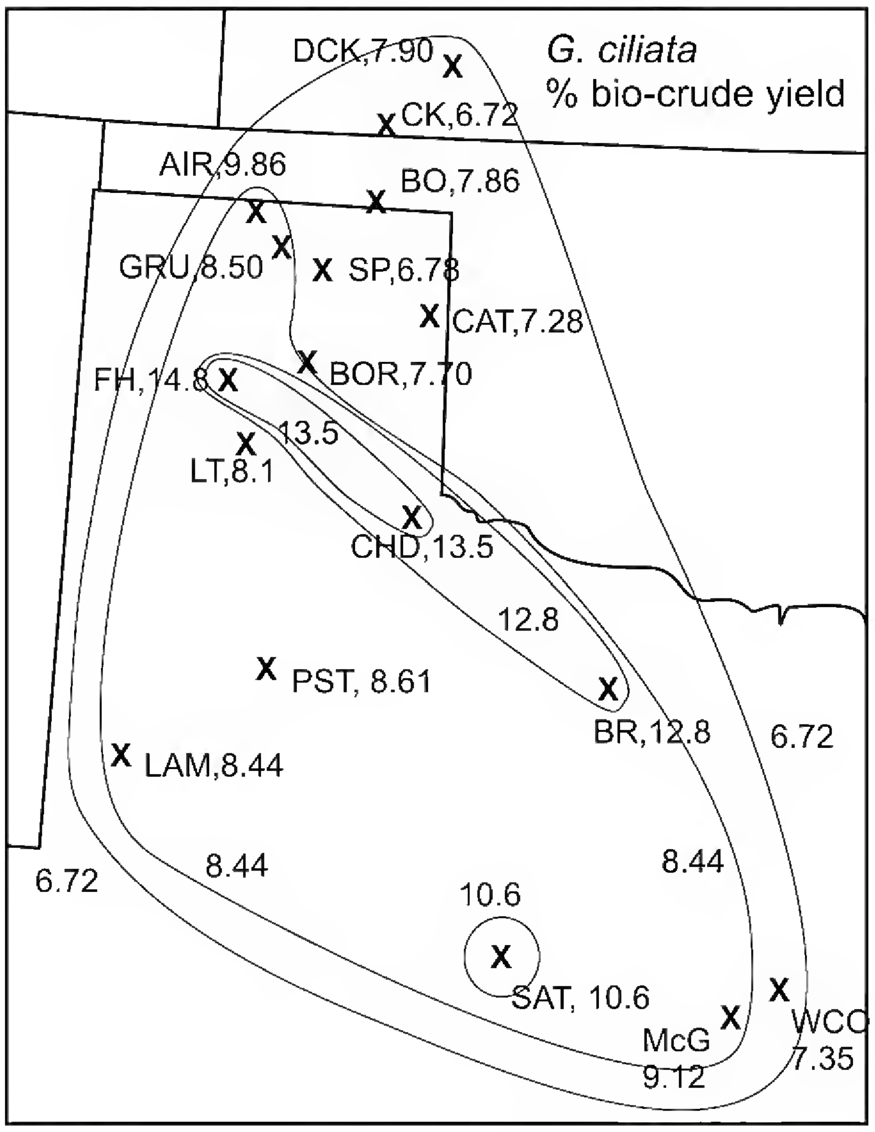

Figure 3. Contoured % bio-crude yields. Number next to the population ID is yield.

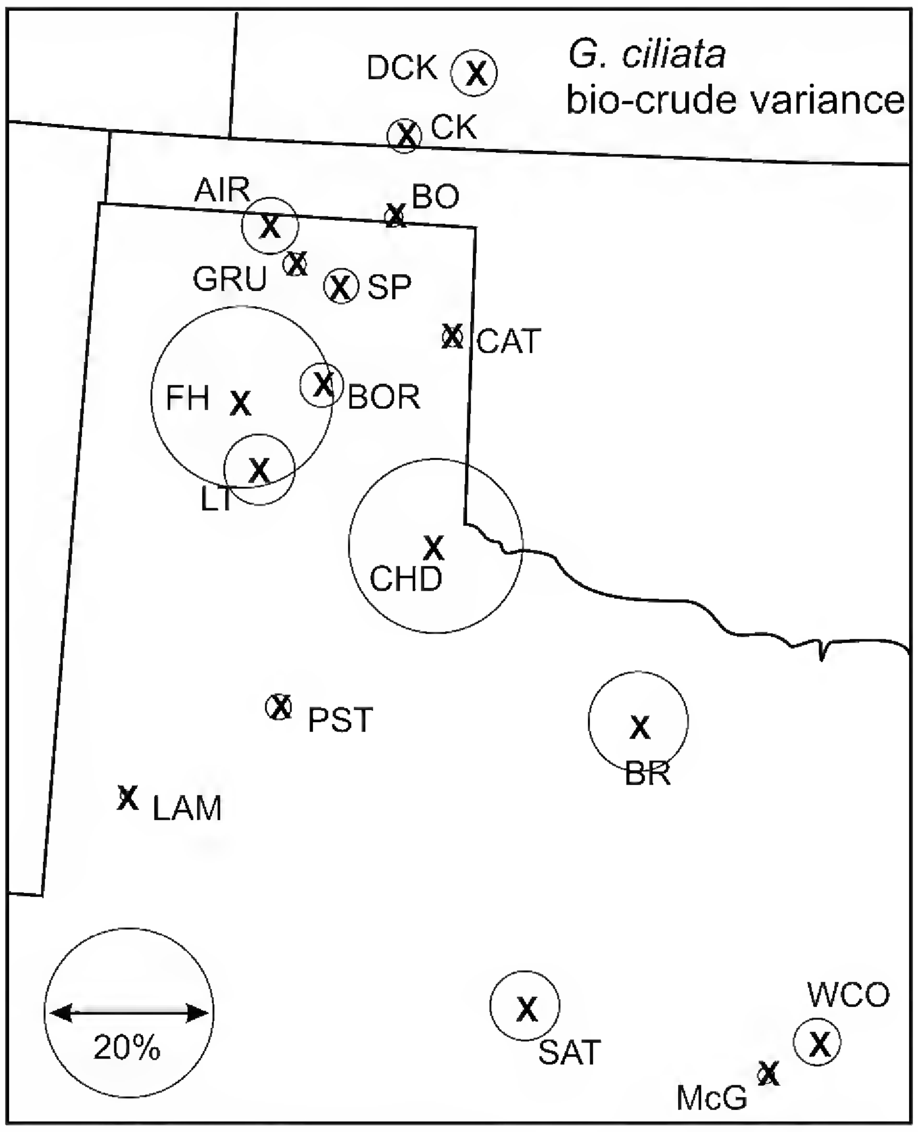

Figure 4. Coefficient of variation (%) per population. The diameters of circles are proportional to CV.

To visualized the geographic trends in population variation of bio-crude, the coefficient of variation (CV) was mapped (Fig. 4). The most variable populations were: Lake Tanglewood (LT, 20.7%), Childress (CHD, 19.56%) and Waco (WCO, 18.6%). The populations with least variation were: Borger, TX (BOR, 5.5%), Lamesa, TX (LAM, 7.6%) and McGregor, TX (McG, 8,8%).

It is instructive to compare the yields from the same plants sampled at different dates. Ten plants were tagged and leaves collected, then, later, additional leaves were collected from the same 10 tagged plants. For the ne Amarillo population (Table 2, top), the average yield dropped from 14.79% (13 Aug) to 8.07% (3 Sept). Yet, the plants were still putting on new buds and healthy. The coefficient of variation (CV) was also smaller in the later sample (Table 2, top). The correlation in yields between the two sampling dates was -0.330. Indeed, one sees essentially no correlation between yields from paired plants in table 2, top.

In contrast, yields from the Bullock Rd., Newcastle (BR) population were not significantly different between 13 Aug and 14 Sept samples (12.84 vs. 13.00%, Table 2 bottom). The CV was slightly higher in the Sept samples (15.7 vs. 17.6%, Table 2, bottom). The correlation in yields between the two sampling dates was +0.758. One sees a correlation between yields from paired plants in table 2, bottom.

It is difficult to explain these conflicting results, except to note that the ne Amarillo population (FH) is at 3,520 ft in a semi-arid grassland with dark loam, whereas Bullock Rd. (BR) is at 1217 ft. is in the rolling plains, red loam area. It may be that the bio-crude extractables have reached a maximum concentration sooner in the much shorter growing season in the high plains (FH) population. Whereas, the bio-crude yield in the longer growing season (BR) population reached a maximum later in the year. To investigate the accumulation of bio-crude throughout the growing season, we are establishing test plots in the high plains (~3,500 ft, Gruver, TX) and lower, rolling plains (~1,200 ft, Newcastle - Graham, TX).

A third comparison was made between population samples (10 plants, randomly chosen) in the Childress (CHD) population. It should be noted that we initially tagged and sampled 10 plants on 30 Aug, but upon return, to re-sample, all the tags had been removed by vandals. So we collected from 10 plants at random. Thus, in Table 3, plants 1-10 are not paired samples. Nevertheless, the averages for bio-crude yields are significantly higher in the 27 Sept samples than in the earlier, 30 Aug samples (11.30 vs. 13.53, Table 3) which is the same trend seen in the BR data (Table 2, bottom). Both of these populations (CHD, 1737 ft, BR, 1217 ft.) are off the caprock and have longer growing seasons than the ne Amarillo population (FH, 3,520 ft.). The CV for the CHD population was larger among the later (27 Sept) samples (13.8%, 19.6%, Table 3). Correlation between sampling periods could not be computed as the samples were not paired.

The distribution of the 166 plants of *G. ciliata* analyzed is shown in Fig. 5. The mode is 7.84% and otherwise normal distribution is skewed to the left. The top 5% yields range from 15.76 to 18.92% bio-crude. Although these data are from leaves, it is interesting to note that Adams et al. (1986) in a very large survey of 832 plants in 614 taxa in the western United States found only *Chrysothamnus paniculatus* and *Parthenium argentatum* with whole plant yields of 16.4%. There is clearly lots of variation in the yields of bio-crude among *G. ciliata* plants, strengthening its promise as a potential new crop for bio-crude.

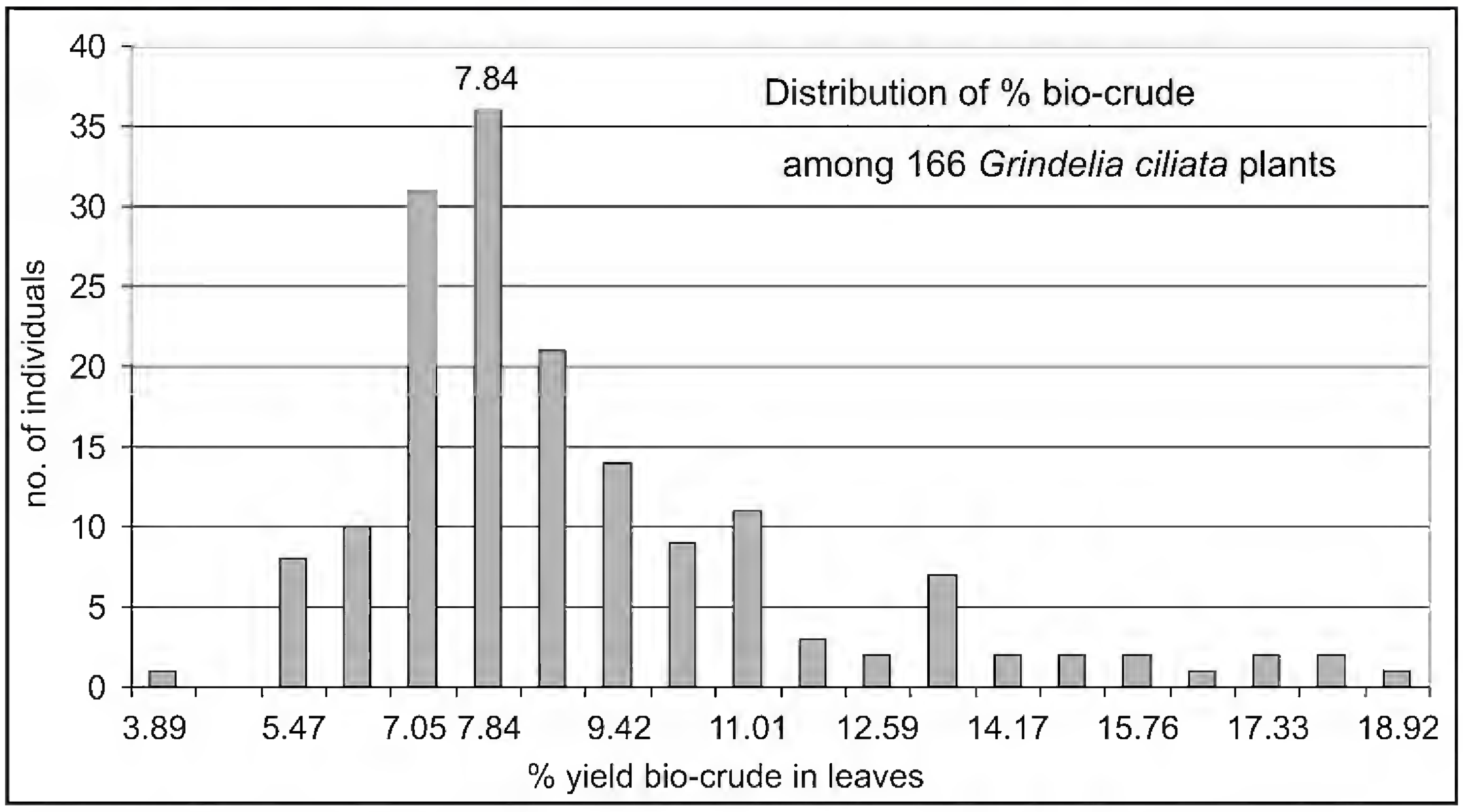

Figure 5. Distribution of % yield of bio-crude in leaves of *G. ciliata* (n= 166).

This first report on variation in bio-crude yields is preliminary because we do no know the optimum time to harvest during the growing season to obtain maximum bio-crude yields. Nevertheless, the results do support the thesis that *G. ciliata* is a promising crop for the production of bio-crude. Additional research utilizing tissue culture cloning and test plots is planned for 2016.

LITERATURE CITED

Adams, R. P., M. F. Balandrin, K. Brown, S. M. Gruel and M. Bagby. 1986. Extraction of liquid fuels and chemicals from higher land plants, Part I: Yields from western United States species. Biomass 9:255-292.

Bartoli, A. and R.D. Tortosa. 2012. Revision of the North American species of *Grindelia* (Asteraceae). Annals of the Missouri Botanical Garden. 98:447-513.

Dunford, M.P. 1964. A cytogenetic analysis of certain polyploids in *Grindelia* (Compositae). Amer. J. Bot. 51(1): 49-56.

Hoffmann, J. J. and S. P. McLaughlin. 1986. *Grindelia camporum*: potential cash crop for the arid southwest. Economic Botany 40: 162-169.

McLaughlin, S. P. and J. J. Hoffmann. 1982. Survey of bio-crude-producing plants from the southwest. Econ. Bot. 36: 323-339.

McLaughlin, S. P. and J. D. Linker. 1987. Agronomic studies on gumweed: seed germination, planting density, planting dates, and biomass and resin production. Field Crops Research 15: 357-367.

Moore, A. J. et al. 2012. Phylogeny, biogeography, and chromosome evolution of the amphitropical genus *Grindelia* (Asteraceae) inferred from nuclear ribosomal and chloroplast sequence data. Taxon 61: 211-230.

Nesom, G. L. , Y. Suh and B. B. Simpson. 1993. *Prionopsis* (Asteraceae: Astereae) united with *Grindelia*. Phytologia 75: 341-346.

Schuck, S. M. and S. P. McLaughlin. 1988. Flowering phenology and outcrossing in tetraploid *Grindelia camporum* Greene. Desert Plants 9: 7-16.

Seinet 2015. http://swbiodiversity.org/seinet/collections/list.php

Steele, R. G. D.; J. H. Torrie. *Principles and procedures of statistics*. McGraw-Hill Book Co.: NY, 1960.

Steyermark, J. A. 1934. Studies in *Grindelia* II: a monograph of the North American species of the genus *Grindelia*. Annals Missouri Botanical Garden 21: 433-608.

Strother, J.L. and M.A. Wetter. 2006. Treatment of *Grindelia*. Flora of North America 20: 424-425.

Timmermann, B. N., S. P. McLaughlin and J. J. Hoffmann. 1987. Quantitative variation of grindelane diterpene acids in 20 species of North American *Grindelia*. Biochemical Systematics and Ecology 15:401-410.

Table 1. Survey of non-polar extractables (bio-crude) from *Grindelia ciliata*. Yields are as percent dry matter (DM) from leaves only. Any averages not followed by a common letter (a,b,c,d,e) are significantly different (P=0.5). CV = population covariance (%).

Population	1	2	3	4	5	6	7	8	9	10	Avg.	CV.
ne Amarillo, TX 8/13	13.88	15.58	18.28	13.65	14.61	18.92	16.98	13.84	10.71	11.43	14.79a	18.3
Childress, TX 9/27 late	13.43	10.08	15.11	12.6	16.87	18.17	13.05	9.69	13.39	12.90	13.53ab	19.6
Bullock Rd., TX	12.29	11.68	11.29	15.57	13.59	11.22	13.13	9.92	16.41	13.28	12.84b	15.7
Santa Anna, TX	7.79	12.14	11.37	11.14	10.46						10.58c	15.8
AIR Products, TX	11.14	11.14	9.16	10.15	10.61	9.84	10.99	6.71	7.97	10.94	9.87cd	15.2
McGregor, TX	9.62	8.32	9.08	8.70	8.55	10.46					9.12cd	8.8
Post, TX	8.40	8.70	10.15	8.47	7.33						8.61cde	11.7
Gruver, TX	7.86	9.54	7.33	8.54	6.87	8.63	9.47	9.80	8.32	8.63	8.50cde	11.3
Lamesa, TX	8.93	7.53	9.28	8.98	7.99	7.63	8.70	8.70	7.78	8.85	8.43cde	7.6
Lake Tanglewood, TX	8.24	9.16	8.02	10.92	10.39	7.30	6.99	7.61	5.25	7.18	8.11de	20.7
ne Amarillo, TX 9/3	7.94	6.83	7.33	9.69	10.38	7.33	7.63	7.56	8.09	7.94	8.07de	13.8
Dodge City, KS	10.70	9.42	6.56	7.00	8.78	7.29	7.67	6.93	6.63	8.03	7.90de	17.2
Beaver R., Oklahoma	6.81	6.78	7.92	7.94	8.64	7.40	9.21	7.23	7.66	9.01	7.86de	10.9
Borger, TX	7.76	8.32	7.02	7.40	7.76	7.10	7.79	7.86	8.17	7.86	7.70e	5.5
Waco,, TX	8.47	9.16	5.34	7.17	5.56	7.72	6.25	7.63	7.01	9.23	7.35e	18.6
Canadian, TX	6.34	6.41	6.77	6.95	7.79	6.34	7.02	8.08	8.53	8.55	7.28e	12.1
Spearmen, TX	6.95	6.95	7.48	7.67	3.89	5.73	7.78	7.02	7.58	6.79	6.78	17.4
Cimarron R., KS	7.25	8.85	5.65	7.51	5.59	6.30	7.48	5.56	5.46	7.56	6.72	17.4

Table 2. Comparison of non-polar extractables (bio-crude) from *Grindelia ciliata* from ne Amraillo, Tx and Newcastle, TX populations for 10 paired plants. ** = P 0.01. CV = population covariance (%).

ne Amarillo, TX 8/13/15 vs 9/3/15 samples	1	2	3	4	5	6	7	8	9	10	Avg.	CV
ne Amarillo, TX 8/13	13.88	15.58	18.28	13.65	14.61	18.92	16.98	13.84	10.71	11.43	14.79	18.3
ne Amarillo, TX 9/3	7.94	6.83	7.33	9.69	10.38	7.33	7.63	7.56	8.09	7.94	8.07**	13.8
Correlation = -0.330												

Newcastle, TX 8/13/15 vs. 9/14/15	1	2	3	4	5	6	7	8	9	10	Avg.	CV
Bullock Rd., TX 8/13	12.29	11.68	11.29	15.57	13.59	11.22	13.13	9.92	16.41	13.28	12.84	15.7
Bullock Rd., TX 9/14	11.39	10.43	10.51	15.10	14.96	11.87	16.23	10.26	14.43	14.68	13.00ns	17.6
Correlation = 0.758												

Table 3. Comparison of non-polar extractables (bio-crude) from *Grindelia ciliata* from a random samples (10 plants) collected on 30 Aug and re-sampled 27 Sept, 2015 at Childress, TX (original tags were removed by vandalism). . * = P 0.05. CV = population covariance (%).

Childress, TX (not paired samples!) 8/30/15 vs. 9/27/15	1	2	3	4	5	6	7	8	9	10	Avg.	CV
Childress, TX 8/30	10.46	11.37	10.08	8.63	13.36	11.91	11.53	9.92	13.36	12.67	11.30	13.8
Childress, TX 9/27e	13.43	10.08	15.11	12.6	16.87	18.17	13.05	9.69	13.39	12.90	13.53*	19.6
Correlation = N. A.												